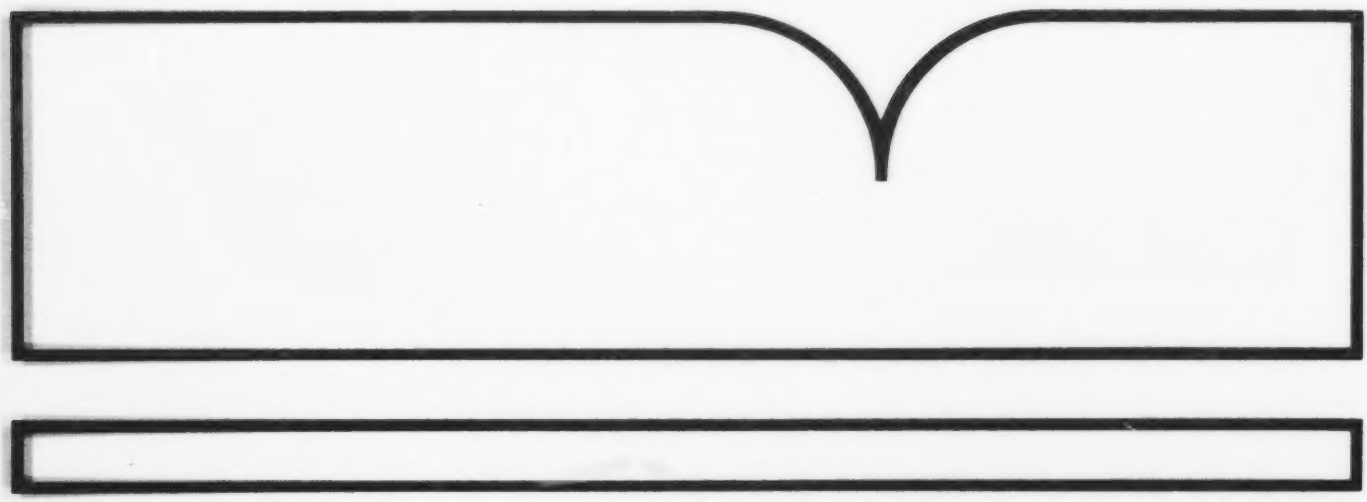
PB87-191516

Environmental Tobacco Smoke: Mutagenic Emission Rates and Their Relationship to Other Emission Factors

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

May 87



U.S. Department of Commerce
National Technical Information Service
NTIS

PB87-191516

EPA/600/D-87/146
May 1987

ENVIRONMENTAL TOBACCO SMOKE: MUTAGENIC EMISSION RATES AND THEIR RELATIONSHIP TO OTHER EMISSION FACTORS

by

Joellen Lewtas and Katherine Williams
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Goran Lofroth
Nordic School of Public Health
Gothenburg, Sweden

Katharine Hammond
University of Massachusetts Medical School
Worcester, MA 01605

Brian Leaderer
Yale University School of Medicine
New Haven, CT 06519

EPA Project Officer
Joellen Lewtas

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-87/146		

4. TITLE AND SUBTITLE
Environmental Tobacco Smoke: Mutagenic Emission Rates and Their Relationship To Other Emission Factors.

5. REPORT DATE
May 1987

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
J. Lewtas*, K. Williams*, G. Lofroth**, K. Hammond***
B. Leaderer@

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
*USEPA, HERL, RTP, NC 27711
**Nordic School of Public Health, Gothenburg, Sweden
***University of Mass., Boston, MA 02125
@Yale University, New Haven, CT 06520

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory, RTP
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The objective of this study was to evaluate the emission rates and exposure concentrations of mutagens, nicotine, and particles from cigarettes. Studies were conducted under controlled laboratory and chamber conditions as well as in personal residences. The mutagenicity of environmental tobacco smoke (ETS) was evaluated in three bioassays using two strains of Salmonella typhimurium. Strain TA98 was used in the standard plate-incorporation and microsuspension histidine reversion assays; and strain TM677 in a microsuspension forward mutation assay. The mutagenicity, expressed either per Ug particle mass or per Ug nicotine, appeared to be a relatively constant factor that did not vary significantly between various cigarette brands. These data are being used to model the emissions of mutagens to predict mutagenic exposure concentrations under various conditions.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	8
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ENVIRONMENTAL TOBACCO SMOKE: MUTAGENIC EMISSION RATES AND THEIR RELATIONSHIP TO OTHER EMISSION FACTORS

Joellen Lewtas and Katherine Williams
USEPA, Research Triangle Park, NC 27711, U.S.A.

Goran Lofroth
Nordic School of Public Health, Gothenburg, Sweden

Katharine Hammond
University of Massachusetts Medical School
Worcester, MA 01605, U.S.A.

Brian Leaderer
John B. Pierce, Yale University School of Medicine
New Haven, CT 06519, U.S.A.

Abstract

The objective of this study was to evaluate the emission rates and exposure concentrations of mutagens, nicotine, and particles from cigarettes. Studies were conducted under controlled laboratory and chamber conditions as well as in personal residences. The mutagenicity of environmental tobacco smoke (ETS) was evaluated in three bioassays using two strains of Salmonella typhimurium. Strain TA98 was used in the standard plate-incorporation and microsuspension histidine reversion assays; and strain TM677 in a microsuspension forward mutation assay. The mutagenicity, expressed either per µg particle mass or per µg nicotine, appeared to be a relatively constant factor that did not vary significantly between various cigarette brands. These data are being used to model the emissions of mutagens to predict mutagenic exposure concentrations under various conditions.

Introduction

The concentration of mutagens associated with particulate matter in indoor air is substantially increased in the presence of tobacco smoke (1, 6, 7). In a residential field study of 10 homes, we found the mutagenicity per cubic meter of air sampled to be highly correlated with the number of cigarettes smoked (4). Recent studies of the mutagenicity of ETS from cigarettes either smoked or smoldering, suggest that the mutagenicity emitted per cigarette is relatively constant (5, 7). Controlled chamber studies showed that the emission rate of nicotine and particles per cigarette from sidestream smoke is also relatively constant (2). The purpose of this study was to determine the mutagenic emission rate and its relationship to respirable particulate matter and nicotine in environmental tobacco smoke (ETS).

Methods

Environmental Tobacco Smoke (ETS) Generation, Sampling and Analysis

The smoking experiments, test chamber, and sample collection methods used are similar to those described in detail by Hammond et al. (2) and are summarized here. ETS was generated by 4 smokers smoking at the rate of 8 cigarettes per hour in a 34-m^3 chamber. In these experiments, the chamber fresh air supply rate was typically 2.6 air changes per hour with a temperature of 19°C and relative humidity of 45%. Particulate matter was collected on 37-mm Teflon-coated glass fiber filters or on 0.5-µm millipore type FH. Vapor-phase nicotine was collected downstream from the filters with a second filter impregnated with sodium bisulfate. The particulate filters were extracted with acetone by sonication and solvent exchanged into dimethyl sulfoxide for bioassay by using a stream of dry nitrogen to evaporate the acetone. The sodium bisulfate-treated filters were extracted and analyzed for nicotine as described by Hammond et al. (2). Equilibrium sampling and calculation of emission rates per gram of tobacco consumed were determined as described by Hammond et al. (2) for nicotine.

Mutagenicity Assays

The principal bioassay employed in this study was a forward mutation assay described originally by Skopek et al. (9) that uses S. typhimurium strain TM677. A microsuspension modification of this assay, described by Lewtas et al. (4), was used to measure mutant frequency (MF) (mutants per 10^6 surviving cells) by plating and counting both 8-azaquanine-resistant (mutant) colonies and surviving colonies. The mutagenicity in this bioassay was compared to that obtained in Salmonella typhimurium strain TA98 that was conducted by either (a) the plate-incorporation assay of protocol of Maron and Ames (8), which will be referred to here as the standard Ames assay, or (b) a microsuspension assay described originally by Kado et al. (3) and modified in our laboratory by using 5x cell concentration; 0.015M phosphate buffer, pH 7.4; and a reduced assay volume that will be referred to here as the microsuspension reverse assay.

All of the bioassays were performed with the addition of Aroclor 1254-induced male Sprague-Dawley rat liver S9 mixture prepared as described in the above-referenced protocols. The initial linear portion of the dose-response curve was used to determine the mutagenicity expressed as either revertants per mg of particles, per m^3 of air, or per cigarette. In all assays both solvent controls (negative) and positive controls were assayed simultaneously as described in the protocols (9, 8, 3).

Results and Discussion

The mutagenicity of ETS was compared across six brands including brands both high and low in tar and nicotine, filtered and unfiltered as shown in Table 1. Despite the significant differences in the cigarette brands, very little difference in the mutagenicity of ETS was observed when measured either per µg of total suspended particulate (TSP) or per m^3.

The mutagenic emission rates shown in Table 1 were also very similar across all six brands and ranged from 22,000-37,000 MF/g tobacco consumed. The emission rate per cigarette would range from 11,000-18,500 MF/cig., assuming that 0.5 g of tobacco is consumed per cigarette smoked.

The ratio of mutagenicity to TSP ranged from 0.7 to 1.4 MF/µg TSP. The TSP and nicotine emission rates for these six brands was determined in a similar series of experiments. The ETS nicotine emission rates (1.6-2.4 mg nicotine/g tobacco consumed) are very similar to that reported earlier by Hammond et al. (2) for four brands. Only Brand E (2.2 mg nicotine/g tobacco) is identical to Brand B (1.8 mg nicotine/g tobacco) in this earlier study. Using this nicotine data from separate experiments, the ratio of MF to nicotine in ETS ranged from 8-16 MF/µg nicotine. This agrees with subsequent experiments not reported here where we have measured both mutagenicity and nicotine on the same samples.

The blank chamber experiment was conducted without smokers present. The TSP and mutagenicity concentrations in this nonsmoking experiment are typical of outside urban air (4).

Table 1: Comparison of mutagenicity of ETS across several brands and mutagenic emission rates compared to nicotine and particles

			Emission Rates			
Cigarette Brand	TSP ($\mu g/m^3$)	Mutant Frequency[a] per m^3	Mutagenicity (MF/g)	TSP[b] (mg/g)	Ratio of MF to TSP (MF/µg)	Ratio of MF to Nicotine[b] (MF/µg)
A*	1177	1178	35,400	35.4	1.0	10
B	1200	1259	25,900	24.7	1.0	16
C†	936	1186	34,600	27.3	1.3	15
D†	905	1280	37,100	26.2	1.4	12
E	1318	907	21,900	32.0	0.7	8
F†	901	1025	30,400	26.5	1.1	-
Blank Chamber	25	91				

[a]Microsuspension forward mutation assay in TM677. Determined from five separate experiments from the linear portion of the dose-response curves.
[b]TSP and nicotine emission rates from these brands were determined in separate chamber studies using the same protocol reported here. The nicotine emission rates in mg nicotine/g tobacco consumed were 2.4 (A), 1.6 (B), 1.6 (C), 2.1 (D), and 2.2 (E).
*Unfiltered cigarette, all others are filtered.
†Low tar and nicotine

Table 2: Comparison of ETS mutagenicity per µg of TSP in different bioassays

ETS Sample	Bioassays		
	Micro TM677 (MF/µg/ml)	Standard TA98 (rev/µg)	Micro TA98 (rev/µg/ml)
Chamber Brand E[a]	0.69	2.9	-
Chamber 2R1[b] Research Cigarette	0.79	1.6	7.0

[a]ETS from studies in 34 m^3 chamber at Yale U.; other data shown in Table 1.
[b]ETS from studies in 13.6 m^3 chamber at EPA using cigarette smoking machine. Ratio of nicotine to TSP was 87 µg nicotine/mg TSP.

ETS is present in indoor air in sufficiently high concentrations to measure the mutagenicity in the standard Ames plate-incorporation assay, which requires approximately 10 times the sample mass of the two microsuspension assays. Table 2 provides a comparison of the mutagenicity of ETS per µg of TSP for several different cigarettes. Although it is possible to use the standard Ames assay to measure the mutagenicity of either sidestream cigarette smoke or ETS, there is one important advantage to using a microsuspension assay. The microsuspension assays require 3-10 times less sample, thereby permitting mutagenicity measurements on samples of less than 1 m^3 of air. These methods will facilitate evaluation of the mutagenicity of personal air samples in human exposure assessment studies.

Disclaimer

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

1. Alfheim I., and Ramdahl T. Contribution of wood combustion to indoor air pollution as measured by mutagenicity in Salmonella and polycyclic aromatic hydrocarbon concentration. Environmental Mutagenesis 6 (1984), 121-130.

2. Hammond, S.K., Leaderer, B.P., Roche, A.C., and Schenker, M. Collection and analysis of nicotine as a marker for environmental tobacco smoke. Atmospheric Environment 21 (1987), 457-462.

3. Kado, N.Y., Langley, D., Eisenstadt, E. A simple modification of the Salmonella liquid incubation assay: Increased sensitivity for detecting mutagens in human urine. Mutation Research 121 (1983), 25-32.

4. Lewtas, J., Goto, S., Williams, K., Chuang, J.C., Petersen, B.A., and Wilson, N.K. The mutagenicity of indoor air particles in a residential pilot field study: Application and evaluation of new methodologies. Atmospheric Environment 21 (1987), 443-449.

5. Ling, P.I., Lofroth, G., and Lewtas, J. Mutagenic determination of passive smoking. Toxicology Letters 35 (1987), 147-151.

6. Lofroth, G., Nilsson, L., and Alfheim I. Passive smoking and urban air pollution: Salmonella/microsome mutagenicity assay of simultaneously collected indoor and outdoor particulate matter. In M.D. Waters, S.S. Sandhu, J. Lewtas, L. Claxton, N. Chernoff and S. Nesnow (Eds.), Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III. New York: Plenum Press, 1983, pp. 515-525.

7. Lofroth, G., and Lazaridis, G. Environmental tobacco smoke: Comparative characterization by mutagenicity assays of sidestream and mainstream cigarette smoke. Environmental Mutagenesis 8 (1986), 693-704.

8. Maron, D.M., and Ames, B.M. Revised methods for the Salmonella mutagenicity test. Mutation Research 113 (1983), 173-215.

9. Skopek, T.R., Liber, H.L., Krolewski, J.J., and Thilly, W.G. Quantitative forward mutation assay in Salmonella typhimurium using 8-azaguanine resistance as a genetic marker. Proceedings National Academy Science 75 (1978), 410-414.